systematic investigations both on the activation process of tyrosine kinases and other families, and in relation to protein-protein interaction mechanisms.

1320-Pos Board B90

Unraveling the Assembly of Large Macromolecular Machines by Integrating Computational Techniques with Experimental Data

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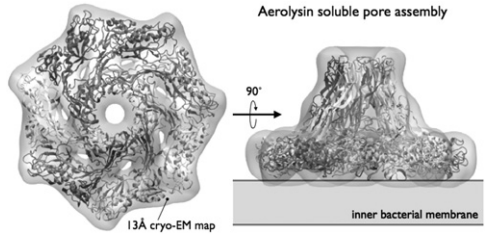
Proteins often assemble in large macromolecular complexes to achieve a specific task. Unfortunately, owing to their size and complexity, the structure of these machines is difficult to be determined at atomistic resolution. Thus, the ability to reliably predict the conformation of multimeric assemblies is desirable. We present a new approach that uses a Particle Swarm Optimization search guided by experimental-based restraints to characterize protein quaternary structure. Moreover, the natural subunit flexibility as extracted from molecular dynamics simulations is explicitly included during model building. This scheme has been successfully used to model of the heptameric soluble and functional forms of pore-forming toxin aerolysin from Aeromonas hydrophila (see Figure). The model is based on the high-resolution X-ray structure of aerolysin monomer and the low-resolution cryo-EM map of the heptamer. The same strategy has been extended to determine the membrane-embedded basal body of the multi-MDa type III secretion system from Yersinia enterocolitica. The method is of general applicability and, coupled with accurate energy functions, can efficiently exploit the spatial restraints derived from a variety of experimental techniques to produce consistent models for the assembly of biological systems.



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Self Assembly Pathways of Surface-Layer Proteins

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Surface layer (S-layer) proteins form a highly ordered crystalline, yet porous, layer on the outmost cell surface of most species of bacteria and archae. S-layers not only provide the cell a layer for protection, they mediate several functions such as cell adhesion, drug resistance and providing a scaffold for mineralization. In order to accomplish these functions on growing cells, newly synthesized S-layer proteins must assemble into crystalline lattices on the cell surface as it changes shape and size. Thus, S-layers provide a robust in vitro model system to test our understanding of the dynamics of self-assembly pathways, as well as a biological scaffold for hierarchical assembly of nanomaterials. The SbpA protein from *Lysinibacillus sphaericus* is a well characterized S-layer protein that forms Ca^{2+} dependent, 2D crystals in solution with square symmetry. Until recently, however, the kinetic pathways of self-assembly have not been identified for this protein. Here we use ensemble techniques to study self-assembly in solution using fluorescence and light scattering. In addition, fluorescence microscopy on lipid bilayers has allowed us to follow assembly of individual crystals in real time. We find that varying protein or Ca^{2+} concentration results in different kinetics of assembly in solution. On lipid bilayers we are able to visualize S-layer protein assemblies, and observe that the rate of self assembly changes with the concentration of Ca^{2+}. Our results, paired with coarse-grained simulations, may provide the first predictive framework for controlling protein self-assembly pathways.

1322-Pos Board B92

Structural Evidence for the Micellar Model of Spider Silk Fibrillogenesis

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Spider silks are outstanding biomaterials with strength and toughness that surpass today's synthetic materials. Despite their desirable properties, very little is currently known about their molecular-level structure and how it relates to mechanical properties. We present the structure of a recombinantly produced uniformly 13-C and 15-N labeled 199aa repeating unit of the Argiope trafisciata aciniform spidroin 1 determined using solution-state nuclear magnetic resonance (NMR) spectroscopy. This protein is a key constituent of egg case sacs, providing both flexibility and strength. The repeat unit has a core 6-helix bundle with an unstructured, ~50 amino acid flexible C-terminal tail. In parallel, atomic force microscopy (AFM) is being used to characterize both mechanical properties and the mechanism of fibril formation of recombinant wrapping silk comprised of multiple repeat units. There are two competing theories for spider silk fibrillogenesis, one involving micellar intermediates, the other involving nucleation and growth by insoluble aggregate formation. Our AFM studies in direct correlation with the structural properties of the repeat unit are strongly suggestive of the micellar model for fibril formation.

1323-Pos Board B93

Origin of Cooperative Relaxation in Polymeric Solids and an Allosteric Assembly

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In certain polymeric solids - such as spin crossover (SCO) solids, and in allosteric assemblies - such as the rigor-state multi-sarcomere assembly, the lattice as a whole may exist in one of two alternative structural states. The elementary unit (protomer) in these two lattice states will differ in shape and/or size. In general, it will be present in a smaller size in one of the lattice states and in a larger size in the other. In recent kinetic studies in polymeric SCO solids, sigmoid-shaped relaxation curves were found, and this shape was regarded as a signature of cooperativity. In our kinetic studies in the allosteric multi-sarcomere assembly, we have also observed sigmoid-shaped relaxation curves.

Here we address the origin of cooperativity in both types of system - polymeric and allosteric. The relaxation experiment on SCO solids begins with photoexcitation of the lattice into a metastable all-large-size state. This metastable lattice is then allowed to relax back to its stable all-small-size state. As individual small-size units begin to appear within the otherwise large-size lattice, they will distort their neighboring large-size units because they will not fit. The misfit-induced distortion of these units will, in turn, reduce the height of their activation barrier - and will thus increase their relaxation rate constant. This rate constant increase represents a self-acceleration effect and is a type of positive feedback. We conclude that positive feedback may play a fundamental role in the origin of cooperative relaxation - both in polymeric SCO solids as well as in an allosteric multi-sarcomere assembly.

1324-Pos Board B94

Viscoelastic Properties of Collagen at the Molecular Scale

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Although extensive studies of collagen viscoelastic properties have been pursued at the macroscopic (fiber/tissue) level, fewer investigations have been performed at the smaller scales, including collagen molecules and fibrils. Here, using an atomistic modeling approach, we perform in silico creep tests of a collagen-like peptide, monitoring the strain-time response for different values of applied external load. The results show that individual collagen molecules exhibit a nonlinear viscoelastic behavior, with a Young's modulus increasing from 6 to 16 GPa (for strains up to 20%), a viscosity of $3.84. \pm 0.38$ Pa s, and a relaxation time in the range of 0.24-0.64 ns. Additionally, the mechanism for molecular sliding between collagen fibrils was studied by shearing the center molecule in a hexagonally packed bundle with varied lateral distance between the molecules. In dry conditions, the central molecule slid with a stick-slip mechanism that corresponded with the breaking and reformation of hydrogen bonds between collagen molecules. This mechanism was observed at varying shear velocities and at different lateral separations between molecules. In water and at small intermolecular lateral distances, the slip-stick behavior was also observed but above the threshold of 13 Å lateral separation, the molecules sheared smoothly in water. Moreover, the average force required to shear remained the same in water as in vacuum, which suggests that the effect of water at this level is to mediate the transfer of load between molecules. Based on our molecular modeling results we propose a simple structural model that describes collagen tissue as a hierarchical structure, providing a bottom-up description of elastic and viscous properties form the properties of the tissue basic building blocks.